GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[x] ORIGINAL [ ] REVISION NO. _____

Project No. G-33-676 GTRI/GIT DATE 8 / 8 /84

Project Director: Dr. Sheldon W. May School/Unit: Chemistry

Sponsor: National Science Foundation

Type Agreement: Grant No. PCM-8402518

Award Period: From 8/1/84 To 1/31/87 * (Performance) 4/30/87 (Reports)

Sponsor Amount:	This Change	Total to Date
Estimated: $		$ 93,000
Funded: $		$ 93,000

Cost Sharing Amount: $ 4,464 Cost Sharing No: G-33-355

Title: "Enzymatic Epoxidation and Oxygen Activation"

DAVID BRIDGES

ADMINISTRATIVE DATA OCA Contact Lynn Boyd x4820

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Defense Priority Rating: _____ Military Security Classification: _____

(or) Company/Industrial Proprietary: _____

RESTRICTIONS

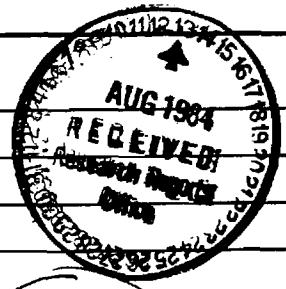
See Attached NSF Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

*includes usual 6-month unfunded flexibility period.



Sponsor I.D. #01.107.000.84.037

COPIES TO:

Project Director
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FORM OCA 4:383

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 02/26/91

Project No. G-33-676 Center No. R5820-0A0

Project Director MAY S W School/Lab CHEMISTRY

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Contract/Grant No. PCM-8402518 Contract Entity GTRC

Prime Contract No.

Title ENZYMATIC EPOXIDATION & OXYGEN ACTIVATION

Effective Completion Date 870131 (Performance) 870430 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	Y	861125
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments98-A SATISFIES THE REQUIREMENT FOR THE PATENT REPORT, SUBMITTED WITH THE FINAL REPORT. NO INVOICE REQUIRED-NSF LOC.

Subproject Under Main Project No.

Continues Project No.

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

NOTE: Final Patent Questionnaire sent to PDPI. 98A

G-33-676

Annual Technical Letter
NSF Grant PCM-8402518

ENZYMATIC EPOXIDATION AND OXYGEN ACTIVATION

Sheldon W. May
School of Chemistry
Georgia Institute of Technology
Atlanta, Ga

The objectives of this research program, focusing on the monooxygenase system from P. oleovorans which carries out epoxidation and hydroxylation of simple aliphatic hydrocarbon substrates (POEHS), are to extend and amplify the mechanistic information we have on the chemical pathway of the reaction, to extend our investigations to the design and evaluation of novel substrates, to focus on interactions at the active site critical to catalysis, and to carry out initial feasibility studies for applying physical techniques to this monooxygenase. The following paragraphs summarize our progress.

Mechanistic and Stereochemical Studies: Work with deutero-olefins 5 has now been completed, and has confirmed the mechanistic view outlined in Scheme I of the proposal. Deuterium migration concommitant with aldehyde formation has now been fully confirmed by mass spectral analysis of products produced from 1,1-diD-1-octene. Furthermore, we successfully completed synthesis of cis-1-D-1-octene and confirmed the 70% inversion of configuration we reported with the trans-1-D-1-octene. Taken together, our finding of corresponding inversion of olefinic geometry together with the D migration accompanying aldehyde formation fully confirm our mechanistic hypothesis, and this work has now been published in a full paper in J.Am.Chem.Soc. Turning to fluorinated substrate analogs, synthesis of 1-F-1-octene, 1,1-diF-1-octene and 2-F-octene have been completed. Characterization of the enzymatic products formed from these substrates is currently being carried out by gc/ms, with CI ms proving essential since complex results are being obtained. We have preliminary evidence for formation of hydroxyoctanoate from both the 1,1-diF- and the 2-F substrates, but the sequence of chemical steps giving rise to this product is still unclear. Model studies on authentic fluorinated epoxides, possible immediate enzymatic products, are currently being carried out. Assay work with both 1-fluoro-octane and 1-H-perfluoro-octane 3 has been completed using the F electrode; both compounds are good substrates. Assay rates show linear dependence on monooxygenase present, absolute dependence on the presence of all components, and a sigmoidal dependence on FeRd, exactly as seen in the standard gc assays. A problem in this work has been the deactivating effect of F substitution which makes product identification studies much more difficult. With our discovery of striking activation of POEHS by imidazole, we now plan to reexamine the F compounds under conditions of much higher turnover. We have found that 1,7-octadiyne is a potent time-dependent inactivator of POEHS. Inactivation exhibits characteristics of mechanism-based irreversible inhibition, and further detailed studies on this and other mechanism-based inactivators are planned. An aspect of the work on these inactivators which we did not anticipate in the proposal is that with the various new catalytic competences we are now finding for POEHS (see below). Mechanism- based inactivators represent tools of choice for demonstrating that these quite distinct substrate classes all undergo reaction at a common active site.

Heteroatom-containing substrates: An extensive amount of work has 5 been done in this area, far beyond what we were able to visualize when the proposal was written. The results have established several heretofore unknown activities for our prototypical NHI monooxygenase, POEHS. First, with thioether substrates (e.g. heptyl methyl sulfide) we have discovered that POEHS readily catalyzes oxygenative conversion $ to both sulfoxide and S-dealkylation products. Sulfide oxygenation exhibits the enzymological characteristics of the normal oxygenative pathway of POEHS, is kinetically facile, and is inhibited by our suicide substrate, 1,7-octadiyne. Studies with a series of thioether substrates revealed that partitioning between the sulfoxide and S-demethylation products is a function of substrate structure. Thus, octyl methyl sulfide is oxidized to the chiral sulfoxide, while p-methoxy phenethyl sulfide gives only the S-demethylation product. S-dealkylation is a process that has not previously been observed with monooxygenases, so we unequivocally demonstrated that this indeed proceeds via an oxygenative pathway by quantitiative trapping of formaldehyde and the thiol; a simple displacement pathway would produce a C-1 product at the oxidation state of methanol. Several types of oxygen-containing substrate analogs have now been investigated. First, we find that terminal oxygenative O-demethylation (e.g. with 1-methoxyoctane) is very readily effected by POEHS, and this is the most kinetically facile activity we have found to date. Several lines of evidence establish that this reaction proceeds via the normal oxygenative POEHS pathway. Strikingly, we have also found that POEHS is indeed capable of non-terminal oxyfunctionalization, as illustrated by the production of either secondary alcohols or ketones via oxygenative O-demethylation of branched alkyl methyl or branched vinyl methyl ethers, respectively. Thus, for example, both 2- and 3-methoxy alkanes readily undergo O-demethylation to the 2- or 3- alcohols, and the vinyl ether, 2-OMe-1-octene, undergoes ogygenative ketonization. In all cases, as expected for an oxygenative process, formaldehyde is also produced stoichiometrically. In related work, we also discovered the facile oxygenation of terminal alcohols to aldehydes by POEHS, again carefully establishing all the enzymological characteristics of the reaction. In our view, these results are highly significant. We have now demonstrated the first example of chiral sulfoxidation by any enzyme system of this type. Coupled with our mechanistic and stereochemical results with olefinic substrates, analysis of the stereochemistry and mechanism of sulfur oxygenation will provide important insight into the chemistry of catalysis. Secondly, our discovery of the heretofore unrecognized competence of POEHS for oxygenation of non-terminal moieties significantly expands the menu of capabilities for this prototypic monooxygenase. This not only allows much more flexibility in the design of new substrates or inhibitors, but it also suggests a greatly expanded synthetic potential for this and other closely related bacterial hydrocarbon hydroxylating enzymes. Our working hypothesis for the mechanism of action of POEHS postulates that the substrate and binding sites of this enzyme are arranged in a manner necessitating oxygen attack at the terminal carbon. It now becomes clear that the reactivity of POM can be directed to positions other than the terminus of a straight chain, given an appropriate balance of binding and inherent reactivity of the functionality undergoing initial electron transfer to the active site metal.

Biochemistry Focusing on the Catalytic Site: Within the past few months we have worked out a new isolation procedure for POM which utilizes FPLC. This will facilitate eventual EXAFS studies where the most difficult biochemical impediment is the requirement for large amounts of enzyme at high concentration. As stated in our proposal, we will draw heavily on our experience with PAH in attempting to apply EXAFS to POEHS. An important finding which we have made recently is that POEHS catalysis is strikingly accelerated by imidazole, presumably via interaction with the active site iron. Stimulation is such that molecules

whose activities were heretofore unmeasurably slow can be clearly seen to undergo reaction. A similar observation has been recently reported by Groves and Watanabe with an iron porphyrin model epoxidation system. This obviously allows us to reexamine reactivity of molecles such as F-olefins which are inherently unreactive toward oxygenation. Characterization and scoping of this imidazole activation and its possible extension to other heterocycles represents an important goal for the future. Furthermore, since we can clearly pick out an imidazole ligand to Fe by EXAFS, as demonstrated in our published work with PCD, a goal in the future will be to see whether the imidazole binds directly to the Fe. If so, it will provide a handle for examining ligation and geometry changes through EXAFS.

School of Chemistry
(404) 894-4002

Georgia Institute of Technology
Atlanta, Georgia 30332
A Unit of the University System of Georgia

November 25, 1986

ella K. Engel
istry Program
l Science Foundation
ton, D.C. 20550

84-02518

ear:

I have just been informed that the grants office at Georgia Tech has no of the enclosed report, which I prepared last fall and sent thorugh to them ailing to you. Therefore, I am sending the enclosed copy to you, and I , in fact, you did not receive the original on account of some clerical

Best regards.

Very truly yours,

Sheldon W. May
Professor of Chemistry

An Equal Educational and Employment Opportunity Institution

...ber 3145-0058

...NAL SCIENCE FOUNDATION
... STREET, NW
...GTON, DC 20550

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...PIENT'S NAME FOR ADDRESS

...TIONAL SCIENCE FOUNDATION
FINAL PROJECT REPORT

PART I - PROJECT IDENTIFICATION INFORMATION

...Program: Biochemistry Program

...Author(s): Morton Steinberg

...Period (Month, Year) From: To:

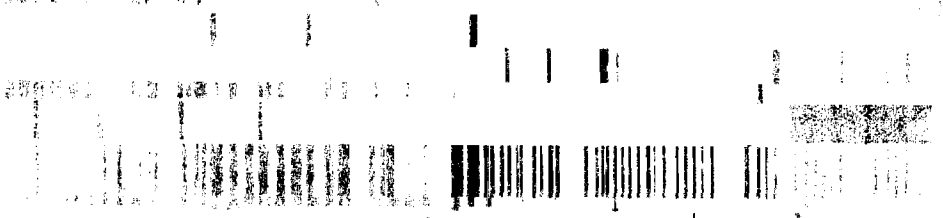
...Author's Mailing Address

...Grant Number

...Project Title

This Packet Contains
NSF Form 98A
and a Return Envelope

Heteroatom-containing substrates: An extensive amount of work has 5 been this area, far beyond what we were able to visualize when the proposal was The results have established several heretofore unknown activities for typical NHI monooxygenase, POEHS. First, with thioether substrates (e.g. ethyl sulfide) we have discovered that POEHS readily catalyzes oxygenative ... to both sulfoxide and S-dealkylation products. ... oxygenation ... the stereochemical characteristics of the normal oxygenative pathway of ... family, and is inhibited by our suicide substrate, ... Studies with a series of thioether substrates revealed that ... between the sulfoxide and S-demethylation products ... fraction of ... structure. Thus, ethyl methyl sulfide is oxidized ... chiral ... while p-methoxy phenethyl sulfide gives only the S-dealkylation ... S-dealkylation is a process that has not previously been observed with ... We unequivocally demonstrated that this indeed proceeds ... an ... pathway by quantitative trapping of formaldehyde ... that initially a ... displacement pathway would produce a C-1 product at the ... level of ... Several types of oxygen-containing substrate analogs have now been ... First, we find that terminal oxygenative O-dem...ylation ... with ... ethanol is very readily effected by POEHS, and this ... the most ... facile activity we have found to date. Several lines of evidence ... that this reaction proceeds via the normal oxygenative ... pathway. ... we have also found that POEHS is indeed capable of ... terminal ... hydroxylation, as illustrated by the production of either ... alcohols ... via oxygenative O-demethylation of branched alkyl ... or ... phenetoles, respectively. Thus, for example, both 2- and ... may ... undergo O-demethylation to the 2- or 3- alcohols ... and ... which, in turn, undergoes oxygenative ketonization. In ... aldehyde is also produced ...



... the ... of the human ... the oxygenation of non-terminal methylene ... a ... for this ... monooxygenase. ... new substrates or inhibitors ... potential for this and ... oxygenating enzymes. Our working ... that the substrate ... assisting oxygen ... the reactivity of POM can be ... that the terminal ... straight chain, given ... the functionality ... the active site metal.

Mechanism-Probing Studies on the Catalytic Site: Within ... we have developed ... procedure for POM which ... These ... ("ghosts") ... were the most difficult ... for ... amounts of enzyme at ... In our proposal, we will draw heavily on our ... with POM to ... important finding ... have made ... is strikingly accelerated by ... with ... Stimulation is ...

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[illegible] AND OXYGEN ACTIVATION

Marvin W. May
School of Chemistry
Georgia Institute of Technology
Atlanta, Ga

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[illegible] Mechanism-based [illegible] gc reaction at [illegible].

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